# Does a new model improve decisions about mismatch-repair genetic testing and Lynch syndrome identification?

Dimitrios Roukos, Michael Fatouros, Epameinondas Tsianos, Angelos Kappas

A new predictive model developed by clinical geneticists in Edinburgh offers a useful tool for physicians making decisions relating to genetic testing, although its clinical application in patients with colorectal cancer for the identification of Lynch syndrome requires caution.

Hereditary nonpolyposis colorectal cancer, also called Lynch syndrome, is caused by a mutation in one of the DNA mismatch-repair (MMR) genes.[1] Pretreatment identification of these carriers among patients with colorectal cancer is critical because it may alter surgical and adjuvant therapeutic decisions. Instead of segmental resection, patients with Lynch syndrome may benefit from prophylactic surgery including total colectomy or proctocolectomy (and hysterectomy with salpingo-oophorectomy for women who have completed childbearing).[2] Ideally, genetic testing of all at-risk patients could identify mutation carriers. The low rate of Lynch syndrome among patients diagnosed with colorectal cancer (2%)[3] and the high costs of testing (about US$3,000 per patient) have led to the development of algorithms based on family history and clinical and pathologic criteria. Currently, based on Bethesda guidelines, tumour immunohistochemistry and microsatellite instability (MSI) are recommended as prescreening tests; patients with abnormal immunohistochemistry or high MSI in tumour analysis are considered for mutational analysis in MMR genes.[4] Even this strategy combining clinical criteria and prescreening misses an appreciable number of mutation carriers, however.[3]

Based on a prospective study in Scotland (see opposite), Barnetson et al. provide a new model for identifying carriers of mutations in the MMR genes *MLH1, MSH2* and *MSH6*. The innovative features of their investigation include population-based recruitment without preselection according to family history or a prescreening with immunohistochemistry or MSI before genetic testing, and a validation approach. This strategy allowed the development of a two-part model predictive of Lynch syndrome, thereby reducing the likelihood of bias. Stage 1 of the model incorporated only clinical variables with significant predictive value: age, sex, location of the tumour, presence of synchronous or metachronous tumours and first-degree relative with colorectal or endometrial cancer. Combining this first stage with immunohistochemistry at stage 2 indicated, with a specificity of 80%, that only 3.4% of patients with colorectal cancer should have mutation testing. This is an excellent finding because immunohistochemistry for MLH1, MSH2, MSH6 and PMS2 protein expression is highly sensitive and associated with several advantages over MSI analysis; it is easily performed, inexpensive, does not require a molecular laboratory and as gene-specific prescreening allows mutational analysis, if abnormal, only for the specific gene.[5] Thus, the overall costs can be reduced substantially. The model is available on the Internet (http://www1.hgu.mrc.ac.uk/Softdata/MMRpredict.php) and is easy to use, helping physicians in making genetic testing decisions for various thresholds of likelihood that a given

Dimitrios Roukos is an assistant professor and Director of the Surgical Oncology & Genomics Research Unit at the Department of Surgery, Michael Fatouros is a professor at the Department of Surgery, Epameinondas Tsianos is a professor and Head of the Department of Internal Medicine and Hepato-Gastroenterology Unit, and Angelos Kappas is a professor and Head of the Department of Surgery, all at the Ioannina University School of Medicine, Ioannina, Greece.
This article was first published in *Nature Clinical Practice Oncology* 2006 vol. 3 no. 12, and is reproduced with permission. www.nature.com/clinicalpractice, doi:10.1038/ncponc0665,
©2006 Nature Publishing Group

patient with colon cancer has a mutation.

The application of this model in clinical practice requires caution, however, because of several study weaknesses. There were only 38 mutation carriers, and participants were under 55 years of age. A recent study, which left out probands and considered only mutation-positive relatives, showed the median age of onset of colorectal cancer to be 61.2 years.[6] Furthermore, mutation in the *PMS2* gene (although admittedly controversial as a cause of Lynch syndrome) was not investigated by Barnetson et al., and the interpretation of many mutations of unknown clinical significance is challenging.

Strategies for identification of healthy carriers and carrier patients are extremely complicated and have not been standardised. It is our professional challenge to provide individualised and efficient management of Lynch syndrome.

*References*

1. HT Lynch, A de la Chapelle (2003) Hereditary colorectal cancer. *N Engl J Med* 348:919–932
2. DH Roukos et al. (2002) Role of surgery in the prophylaxis of hereditary cancer syndromes. *Ann Surg Oncol* 9:607–609
3. H Hampel et al. (2005) Screening for the Lynch syndrome (hereditary nonpolyposis colorectal cancer). *N Engl J Med* 352:1851–1860
4. NCCN Clinical Practice Guidelines in Oncology, version 1.2006: colorectal cancer screening.[http://www.nccn.org/professionals/physician_gls/PDF/colorectal_screening.pdf] (accessed 16 August 2006)
5. HJ Lenz (2005) First Amsterdam, then Bethesda, now Melbourne? *J Clin Oncol* 23:6445–6449
6. H Hampel et al. (2005) Cancer risk in hereditary nonpolyposis colorectal cancer syndrome: later age of onset. *Gastroenterology* 129:415–421

# Synopsis

*RA Barnetson, A Tenesa, SM Farrington, et al. (2006)* **Identification and survival of carriers of mutations in DNA mismatch-repair genes in colon cancer.** *N Engl J Med 354:2751–2763*

**Background.** It is important to identify any mutations in germline mismatch-repair (MMR) genes at the time of diagnosis of colorectal cancer, as this affects management. Pragmatic and cost constraints often lead to 'prescreening' for microsatellite instability (MSI) or DNA MMR proteins, or both, being performed to select patients for genotyping, but this can cause mutations in DNA MMR genes to be missed.

**Objective.** To undertake mutational analysis of germline DNA MMR genes without considering the family history or results of tumour testing among cases of early-onset colorectal cancer in order to construct a predictive model.

**Design and intervention.** In a prospective, population-based series, all patients in Scotland with early-onset colorectal cancer diagnosed between February 1999 and July 2003 were identified and invited to participate within a few weeks after diagnosis. Family history was established and tumour and blood samples were taken. Tumour samples were analysed by immunohistochemistry and tests for MSI, and staged using Tumour–Node–Metastasis criteria and Dukes' classification. Germline DNA from leukocytes was examined for *MLH1, MSH2* and *MSH6* mutations. A two-stage model to detect the presence of germline mutations of DNA MMR genes was constructed using logistic regression. Stage 1 used exclusively clinical variables to identify subgroups more likely to include carriers, and stage 2 involved MSI and immunohistochemistry testing. The model was validated in a separate retrospective series of patients.

**Outcome measure.** Survival was analysed according to genotype using Kaplan–Meier analysis.

**Results.** Among the 870 patients originally studied, 38 mutations were identified (4%): 15 mutations in *MLH1*, 16 in *MSH2* and 7 in *MSH6*. Carrier frequencies were higher in men than in women (6% vs 3%; $P<0.04$). Most carriers would have been identified using Bethesda criteria, but only 42% had characteristics concordant with the Amsterdam criteria. Clinical variables that were predictive of mutational status were having a first-degree relative with colorectal cancer ($P<0.001$) or endometrial cancer ($P=0.006$), age ($P<0.001$), sex ($P=0.03$), tumour location ($P<0.001$), and the presence of synchronous or metachronous tumours ($P=0.001$). Addition of immunohistochemistry in stage 2 of the model provided a sensitivity of 62% (95% CI 0.46–0.77%) and a positive predictive value of 80% (95% CI 0.66–0.95%). Addition of immunostaining of biopsy specimens in the 17% of the population enriched for mutation carriers indicated a requirement for mutational analysis in only 3.4% of all cases. In the validation group of 155 patients, mutational analysis of germline DNA identified 19 mutations in *MLH1*, 13 in *MSH2* and 3 in *MSH6* (35 mutations overall; 23%). Survival did not differ between carriers and noncarriers over 2,938 patient-years of follow-up.

**Conclusion.** The authors suggest that this model would be a highly efficient means of identifying patients who should receive mutation testing.

*Acknowledgement:*The synopsis was written by Petra Roberts, Associate Editor, *Nature Clinical Practice*.